

Boehringer Mannheim GmbH
4340/00/

**Stable lyophilized pharmaceutical preparations of
monoclonal or polyclonal antibodies**

The invention concerns lyophilized pharmaceutical preparations of monoclonal or polyclonal antibodies which contain a sugar or amino sugar, an amino acid and a surfactant as stabilizer. In addition the invention concerns a process for the production of these stable lyophilisates as well as the use of a sugar or amino sugar, an amino acid and a surfactant as stabilizers of therapeutic or diagnostic agents containing antibodies.

The production of immunoglobulins in particular monoclonal and polyclonal antibodies, for therapeutic and diagnostic purposes is nowadays of major and continuously increasing importance.

The use of antibodies as pharmacological agents has been already known for a long time and comprises numerous applications. Hence antibodies have been for example used successfully for tetanus prophylaxis, to combat pathogenic microorganisms or to neutralize their toxins and also for poisoning by snake venoms.

If the antigen involved in the disease mechanism has been identified, which is the case for numerous infectious and some oncological indications for antibody therapy, one utilizes the specificity of the antibodies for the therapy.

09/308223 "081299
56280 E2280E60

In clinical and preclinical studies antibodies are presently used to lower the cholesterol level, to influence the angiotensin/renin system and in autoimmune diseases such as for example lupus, autoimmune encephalitis, multiple sclerosis, polyarthrititis and autoimmune myasthenia gravis.

Additionally of major therapeutic importance is their application to counteract intoxications by low molecular substances such as e.g. the Fab fragments of anti-digoxin antibodies when used for intoxications by digoxin or the cardiac glycosides digitoxin and ouabain. Moreover antibodies are used in the diagnostic field to identify, purify and determine the content of proteins.

Genetic engineering which revolutionized the production of monoclonal antibodies in cell cultures in the second half of the 70's and in the 80's has greatly advanced the preparation of antibodies.

In order to fulfil these diverse applications it is necessary to have pharmaceutical preparations of monoclonal and polyclonal antibodies that are stable on storage. There are a number of publications relating to liquid formulations or lyophilisates of special antibodies. Thus for example liquid formulations of antibodies are described in EP 0 280 358, EP 0 170 983, WO 89/11298, EP 0 352 500 and JP 63088197.

According to EP 0 280 358 dextran is added to the antibody solution to stabilize it towards certain hormones by which means it was possible to achieve a stability of over nine months. According to EP 0 170 983 hydrolysed ovalbumin is added to stabilize a

09308223-084290

thermolabile monoclonal antibody when heated and as a result the antibody could still be used after storage at 45°C for 7 days. Polyhydroxy alcohols (e.g. glycerol, inositol, polyvinyl alcohol) or sugars (e.g. sucrose and glucose) or glycitols (e.g. sorbitol, mannitol) are known from JP 63088197 as further stabilizers for liquid formulations. WO 89/11298 demonstrates the use of maltose in a phosphate buffer containing sodium chloride as a further method for the liquid stabilization of monoclonal antibodies. EP 0 352 500 describes polyethylene glycol 4000 and 3-propiolactone for the liquid stabilization of monoclonal antibodies.

However, in general liquid formulations are not an optimal solution due to storage stability since the proteins or aggregates thereof may precipitate in time during storage, at increased temperatures, when transported through different climatic zones or by improper storage (e.g. interruptions in the cool chain) and the solutions may thus have a reduced protein content and become turbid. Hence, a problem-free use of the solutions cannot be guaranteed in these cases.

In contrast in the case of a lyophilisate formulation the removal of water minimizes the formation of degradation products (e.g. by deamidation and hydrolysis) and aggregate formation. The residual content of water (bound water) can contribute to the stability particularly in the presence of sugars (Hsu et al. Dev. Biol. Stand. 1991, 74: 255-267 and Pikal et al., Dev. Biol. Stand. 1991, 74: 21-27).

Lyophilisate formulations with special antibodies as active substances are also known from the literature but

they do not give consistent advice about the problem of stabilization. Hence in WO 93/00807 the stabilization of biomaterials is described such as human proteins, growth hormones, interleukins, interferons, enzymes and also monoclonal and polyclonal antibodies by a two component system consisting of cryoprotective agents (e.g. polyethylene glycols) and a compound which can form hydrogen bridges with proteins. However, a disadvantage of these preparations is that the addition of high molecular compounds such as polyethylene glycols can lead to an accumulation in the body with potentially toxic side-effects if there is no biodegradation. Furthermore, as is well-known, polymers can act as antigens depending on their molar mass.

Lyophilisates of a monoclonal antibody that is labile when frozen are stabilized for one year according to JP 60146833 by the addition of albumin (human, horse or bovine albumin). Human serum albumin (HSA) is also described in EP 0 303 088 in combination with a carbohydrate (e.g. dextrose, sucrose or maltose) to stabilize a monoclonal antibody for the treatment of *Pseudomonas aeruginosa* infections.

Human serum albumin (in combination with sugars and amino acids) is also the principle by which monoclonal antibodies are stabilized in EP 0 413 188. In JP 01075433 a mixture of human serum albumin, mannitol and polyethylene glycol is used to stabilize a human monoclonal antibody as a lyophilisate. A further example of the use of macromolecules such as e.g. polyethylene glycols and protecting proteins such as human serum albumins to stabilize gamma-globulins during lyophilization is shown in WO 84/00890.

09308223-081299

In WO 93/01835 Hagiwara et al. describe the stabilization of a human monoclonal antibody by lyophilization with mannitol and glycine in a solution containing sodium chloride and phosphate buffer. Stable preparations are obtained with regard to freezing, lyophilization and reconstitution.

Draber et al. (J. Immun. Methods, 1995, 181:37-43) were able to produce a stable formulation of monoclonal IgM antibodies from the mouse at 4°C by the addition of trehalose alone and in combination with polyethylene glycol 8,000. However, the antibodies are only stable for 14 days at 50°C. Using other monosaccharides or disaccharides alone such as e.g. sucrose, maltose, lactose or galactose it is not possible to stabilize these antibodies.

A monoclonal antibody from the mouse is converted into a stable lyophilisate in WO 89/11297 using a carbohydrate (maltose) and a buffer in the acid range (acetate buffer). In this case a disadvantage is the limitation to buffering in an acid range.

Polymeric gelatin as a freezing protectant and stabilizer in a lyophilisate is used in WO 92/15 331. The stabilization is also achieved in combination with a carboxylic acid (e.g. citric acid) or a salt thereof as well as with a primary, secondary or tertiary alcohol or an amino acid in a pH range of 6.8 to 8.1.

In a whole series of the aforementioned publications pharmaceutical additives or auxiliary substances are proposed as stabilizers which are not acceptable from a medical point of view. Hence polymers (such as PEG or

662T80" E2280E50

gelatin) and proteins (such as serum albumins) pose a certain risk due to their origin and their physico-chemical properties and can trigger allergic reactions even to the point of an anaphylactic shock. Proteins of human or animal origin as well as proteins obtained from cell cultures carry the residual risk of viral contaminations. However, other protein-like contaminations which are difficult to detect analytically can cause immunological reactions in humans due to their properties.

The addition of polymeric compounds such as e.g. polyethylene glycols (PEG) or gelatin can lead to an accumulation in the body with potentially toxic side-effects if there is no biodegradation. Polymers may also have antigenic properties depending on their molar mass. Also it is difficult to ensure the purity of polymers due to the catalysts used in their production or the presence of monomers and other polymer fragments. The use of polymers in pharmaceutical forms of administration, especially in drug forms that can be administered subcutaneously, should be avoided if another type of stabilization is possible.

In contrast the use of sugars alone without other additives does not always ensure an adequate protective effect when the antibodies are lyophilized.

Hence the object of the invention was to provide a stable pharmaceutical preparation of monoclonal or polyclonal antibodies that is essentially free of the above-mentioned polymers or proteinaceous pharmaceutical auxiliary substances. This applies particularly to those antibodies which are labile towards freezing and thawing

00308223 081299 662180 22280660

processes or towards multiple freezing and thawing processes.

Surprisingly it was found that stable pharmaceutical lyophilisates of monoclonal or polyclonal antibodies are obtained if these contain sugar or amino sugar, an amino acid and a surfactant as additives. The lyophilisates are preferably composed of a) the antibody, b) a sugar or amino sugar, c) an amino acid, d) a buffer for adjusting the pH value and e) a surfactant. Those lyophilisates are particularly preferred which only contain a single or two different amino acids.

These preparations are physiologically well tolerated, have a relatively simple composition and can be dosed exactly. In addition they are stable i.e. they exhibit no detectable degradation products or protein aggregates when subjected to multiple freezing and thawing processes as well as on longer storage. The lyophilisates can even be stored without stability problems at refrigerator temperature (4 - 12°C) or even at room temperature (18 - 23°C) over a time period of at least three months, preferably at least six months and in particular of at least one to two years. Furthermore they are also stable when stored at higher temperatures (for example up to 30°C). The storage stability is for example exhibited by the fact that during the said storage period only a very small number of particles can be detected when the lyophilisates are reconstituted in the containers with water for injection purposes or with isotonic solutions. In particular the containers have fewer than 6000 particles with a particle size of more than 10 μm and/or less than 600 particles with a particle size of more than 25 μm . The solutions prepared in this manner are stable over a time period of about up

09308223 081299

to five days, preferably up to three days.

The fact that the preparations protect against freezing due to the selected combination of additives is particularly advantageous. Hence, in particular this enables a lyophilization at temperatures down to -45°C without impairing the stability of the antibodies. In addition the lyophilisates containing the combination of additives according to the invention are also stable for a long period and during storage even at relatively high temperatures. Especially compared to conventional formulations, they exhibit no particle formation after reconstitution with water, i.e. the solutions are essentially free of turbidities.

The preparations according to the invention have the additional advantage of being essentially free of protein-like or polymeric auxiliary substances the use of which may be problematic from a medical point of view. Due to the fact that liquid therapeutic or diagnostic agents containing antibodies with a pH value of about 5 to 8, preferably with a pH value of 6.0 - 7.4 (pH value of blood 7.2 - 7.4) can now be prepared by dissolving lyophilisates, they have the additional advantage of being well-tolerated and can be administered substantially free of pain. This is above all important for subcutaneous administration since in this case intolerances develop more easily than when administered intravenously.

The formulations according to the invention can in general be produced in clinically relevant concentration ranges of the antibody for example of up to 20 mg/ml preferably up to 10 mg/ml. Preferred concentration

ranges are concentrations above 0.01 mg/ml in particular above 0.05 and 0.1 mg/ml. In particular concentration ranges of 0.05 - 10 mg/ml or 0.1 - 5 mg/ml for example about 5, 8 or 10 mg/ml are used. The injection volumes of the solutions used are less than 2 ml preferably about 1 ml in the case of subcutaneous or intravenous injections. Small injection volumes are particularly advantageous for subcutaneous administration since they only cause slight mechanical irritation in the subcutaneous tissue. Basically the solutions are also directly suitable as additives to infusion solutions or as infusion solutions. If they are used as additives to infusion solutions the concentration of the antibodies is at higher levels, for example up to 10 mg/ml. These concentrated solutions of the antibodies are then added to conventional infusion solutions so that the concentration of the antibody in the infusion solution to be administered is in the therapeutically relevant range. This range is normally 0.001 - 0.5 mg/ml.

The pharmaceutical single forms of administration can either be present as ready-to-use infusion solutions or injection solutions or also as lyophilisates. If the pharmaceutical preparations are used in the form of lyophilisates, the single dose containers, for example glass ampoules with a volume of 10 ml, contain the antibody in amounts of 0.1 - 500 mg, preferably 10 - 100 mg depending on the respective therapeutically relevant dose of the antibody. The lyophilisate optionally contains additional conventional pharmaceutical auxiliary substances. The lyophilisate is dissolved with an appropriate amount of reconstitution solution and can then either be used directly as an injection solution or as an additive to an infusion solution. If it is used as an additive to infusion

09308223 "081299

solutions, the lyophilisate is usually dissolved with about 10 ml of a reconstitution solution and added to a physiological saline solution (0.9 % NaCl) of 250 ml. The resulting infusion solution is then usually administered to the patient within about 30 minutes.

The sugars used according to the invention can be monosaccharides, disaccharides or trisaccharides. Glucose, mannose, galactose, fructose and sorbose come into consideration as monosaccharides. Sucrose, lactose, maltose or trehalose come into consideration as disaccharides. Raffinose is preferably used as the trisaccharide. According to the invention sucrose, lactose, maltose, raffinose or trehalose are especially preferably used. Instead of maltose it is also possible to use the stereoisomeric disaccharides cellobiose, gentiobiose or isomaltose.

Those monosaccharides are generally referred to and used as amino sugars which have an amino ($-NH_2$, $-NHR$, $-NR_2$) or an acylated amino group ($-NH-CO-R$) instead of a hydroxy group. For this glucosamine, N-methyl-glucosamine, galactosamine and neuraminic acid are particularly preferred according to the invention. The sugar content or amino sugar content is for example up to 2000 mg, preferably up to 1000 mg especially up to 800 or up to 500 mg per single form of administration. Amounts of more than 10, 50 or 100 mg come for example into consideration as the lower limit for the sugar content. Preferred ranges are 200 - 1000 mg, especially 400 - 800 mg. The stated quantities per single form of administration refer to single forms of administration which are marketed as lyophilisates. Such lyophilisates are preferably filled into injection bottles with a volume of 10 ml. After dissolution of the lyophilisates

09308223-084299

with a reconstitution solution of 10 ml, liquid forms of administration are obtained which can be administered directly. The sugar concentration in these injection solutions is up to 200 mg/ml, preferably up to 100 mg/ml based on the amounts stated above of the sugars used.

The amino acids used according to the invention can be basic amino acids such as arginine, lysine, histidine, ornithine etc., the amino acids preferably being used in the form of inorganic salts thereof (preferably in the form of phosphoric acid salts i.e. as amino acid phosphates). If free amino acids are used, the desired pH value is adjusted by adding a suitable physiologically tolerated buffer substance such as e.g. an inorganic acid in particular phosphoric acid, sulphuric acid, acetic acid, formic acid or salts thereof. In this case the use of phosphates has the particular advantage that particularly stable lyophilisates are obtained. It has proven to be advantageous when the preparations are essentially free of organic acids such as e.g. malic acid, tartaric acid, citric acid, succinic acid, fumaric acid, etc. or the corresponding anions (malates, oxalates, citrates, succinates, fumarates, etc.) are not present.

Preferred amino acids are arginine, lysine or ornithine. In addition it is also possible to use acidic amino acids such as glutamic acid and aspartic acid or neutral amino acids such as e.g. isoleucine, leucine and alanine or aromatic amino acids such as e.g. phenylalanine, tyrosine or tryptophan. The amino acid content in the aqueous preparations according to the invention is up to 100 mg/ml, preferably up to 50 mg/ml or up to 30 mg/ml. The lower limit may for example be concentrations above 1, 5 or 10 mg/ml. Preferred concentrations are for example in the range of 3 - 30 mg/ml or 10 - 25 mg/ml.

662 FEB 22 1960

If the corresponding forms of administration are marketed as lyophilisates, these lyophilisates are preferably made available in injection bottles (volumes of for example 10 ml). Such single forms of administration contain the amino acids in amounts of up to 1000 mg, preferably up to 500 mg or up to 300 mg.

Surfactants which come into consideration are all surfactants that are usually used in pharmaceutical preparations preferably polysorbates and polyoxy-ethylene-polyoxypropylene polymers such as e.g. Tween®. Low amounts of surfactant of 0.05 to 0.5 mg/ml preferably 0.1 mg/ml are sufficient to stabilize the antibodies. In the above-mentioned single forms of administration the amount of surfactants is 0.5 - 5 mg in the case of a lyophilisate that is filled into an injection bottle of 10 ml.

The stabilization of antibodies achieved by the said additives relates in principle to all known monoclonal and polyclonal antibodies and their Fab fragments. Humanized antibodies and modified antibodies (cf. e.g. US 5,624,821; EP 0 592 106; PCT/EP96/00098) are preferably used. The molecular weight of the antibodies is 50 kDa-200 kDa per monomer unit, in particular the molecular weight is about 80 - 150 kDa. In particular antibodies to the hepatitis B virus (cf. WO 94/11495), to AIDS viruses, cytomegalo viruses, meningoencephalitis viruses (FSME), rubella viruses, measles viruses, rabies pathogens, Pseudomonas aeruginosa bacteria, varicella-zoster viruses, tetanus pathogens, van Willebrandt factor (cf. WO 96/17078), NGFR (nerve growth factor receptor), PDGFR (platelet derived growth factor receptor: Shulman, Sauer, Jackman, Chang, Landolfi, J. Biol. Chem. 1997, 272(28): 17400-4), selectin, in particular E-selectin,

L-selectin (cf. Takashi et al., Proc. Natl. Acad. Sci. USA 1990, 87: 2244-2248; WO 94/12215) or P-selectin; integrins or diphtheria pathogens can be stabilized according to the invention. The antibody concentration can preferably be up to 8 mg/ml. It is preferably for example 0.05 - 2 mg/ml. The amount of antibody in the single form of administration, for example in a lyophilisate in an injection bottle of 10 ml, is up to 100 mg preferably up to 80 mg, 50 mg, 20 mg or 10 mg. The concentration of the antibodies after reconstitution of the lyophilisates with a volume of 10 ml is in the range of 1 - 10 mg/ml, preferably at 5 - 8 mg/ml.

In addition to the said additives, sugar, amino acid and surfactant, the lyophilisates according to the invention can contain physiologically tolerated auxiliary substances from the group comprising acids, bases, buffers or isotonizing agents to adjust the pH value to 5 to 8, preferably 6.0 to 7.4. The buffer capacity of the preparations is adjusted such that when the lyophilisates are dissolved with standard reconstitution solutions such as for example water for injection purposes the buffer concentration is in the range between 10 - 20 mmol/l preferably at about 15 mmol/l.

The order of addition of the various auxiliary substances or of the antibody is largely independent of the production process and is up to the judgement of a person skilled in the art. The desired pH value of the solution is adjusted by adding bases such as for example alkali hydroxides, alkaline earth hydroxides or ammonium hydroxide. Sodium hydroxide is preferably used for this. The desired pH value can in principle be adjusted by adding basic solutions. In this sense salts of strong bases with weak acids are generally suitable such as

09308223 031299

sodium acetate, sodium citrate, di-sodium or sodium dihydrogen phosphate or sodium carbonate. If the pharmaceutical solution of auxiliary substances has a basic pH value it is adjusted by titration with an acid until the desired pH range has been reached.

Physiologically tolerated inorganic or organic acids come into consideration as acids such as for example hydrochloric acid, phosphoric acid, acetic acid, citric acid or conventional solutions of substances which have an acidic pH value. In this sense preferred substances are salts of strong acids with weak bases such as e.g. sodium dihydrogen phosphate or disodium hydrogen phosphate. The pH value of the solution is preferably adjusted with phosphoric acid or an aqueous sodium hydroxide solution.

In order to produce well-tolerated parenteral drug forms it is expedient to add isotonizing auxiliary substances if isotonicity cannot be already achieved by the osmotic properties of the antibody and the additives used for stabilization. Non-ionized well-tolerated auxiliary substances are used above all for this. Salts such as NaCl should, however, only be added in small amounts, in particular a value of 30 mmol/l in the final injection or infusion solution for administration should not be exceeded.

In addition the pharmaceutical preparations can contain further common auxiliary substances or additives. Antioxidants such as for example glutathione or ascorbic acid or similar substances can be added.

For the production of the lyophilisates the aqueous pharmaceutical solutions which contain the antibody are

0930323 081239 662130 22230360

firstly produced. A buffered antibody solution containing sodium chloride is preferably prepared. This antibody solution is admixed with an aqueous solution containing the additives sugar, amino acid and surfactant during which the pH value is adjusted with an acid or base to 5 to 8. Phosphoric acid or phosphate salts and sodium chloride are added in such amounts that the previously defined concentrations are obtained. Subsequently it is sterilized by filtration and the solution prepared in this manner is lyophilized.

The invention also enables unstable aqueous solutions containing antibodies that are sensitive to freezing to be also converted by means of freeze-drying into stable preparations that are also stable at high temperatures without impairing the quality.

A further advantage of the lyophilisates according to the invention is that, in addition to avoiding damage to the antibodies during freezing, they also exhibit no reduction in the antibody content and no aggregate formation or flocculation even after a long-term storage at 50°C. They are thus stable with regard to antibody content and purity. The formation of particles is prevented which is exhibited by the low values for turbidity after reconstitution of the lyophilisates with water for injection purposes.

The invention is elucidated in more detail in the following on the basis of examples of application.

Examples 1 to 10 show in which manner the lyophilisates according to the invention can be formulated, produced and examined with regard to antibody stability.

00308223 1081299

Comparative experiments without auxiliary substances or with sucrose alone or with mannitol as a substitute for the sugar component or with the amino acid component alone or only the sugar or amino acid component without the surfactant show that the choice of the combination of additives according to the invention is essential for achieving a stable formulation. Sucrose alone, amino acid alone or both components without surfactant lead to unstable formulations.

The formulations according to the invention are insensitive to freezing and it is possible to completely omit polymers or proteins that are regarded as being toxic such as polyethylene glycols, gelatin, serum albumins. In the case of the surfactants only relatively small amounts of physiologically well-tolerated surfactants are present.

The antibody to HBV used in the following application examples is a recombinant human monoclonal antibody (MAB) from a murine cell. It has a molecular weight of about 147 kDa and is directed towards the hepatitis B surface antigen (HBsAg) of the hepatitis B virus. The monoclonal antibody recognizes the a-determinant of the HBsAg which is constant in almost all known variants of the virus. This antibody can for example be used for the following medical indications: treatment of chronic hepatitis for which there has previously not yet been a satisfactory treatment method; treatment of passive immunoprophylaxis in HBsAg-positive liver transplant patients. In central and northern Europe and the USA up to 2 % of the population are carriers of the hepatitis B virus, in southern Europe up to 3 %, in Africa and the Far East it is 10 - 15 %. A consequence of this chronic infection is that the risk of developing hepatocellular

carcinoma is increased by 100-fold, 40 % of the virus carriers die as a result of this infection.

Antibodies to L-selectin, the NGF receptor or the PDGF receptor can be preferably used as antibodies within the sense of the invention.

Example 1 shows the properties of an aqueous solution of a monoclonal antibody to hepatitis B virus (MAB HBV; INN name: Tuvirumab) containing phosphate buffer and sodium chloride at pH = 5, pH = 6.5 and pH = 8 after freezing and thawing. It shows that freezing and thawing damages the monoclonal antibody.

Example 2 demonstrates the possibility of stabilizing a preparation according to the invention with sucrose or maltose or an amino sugar (N-methylglucosamine or galactosamine) and arginine phosphate and Tween 20 with a concentration of the antibody of 2 mg/ml i.e. 2 mg in the lyophilisate.

The same preparation as in example 2 is shown in example 2a except that the antibody concentration is 8 mg/ml. It can be seen from examples 2 and 2a that the combination of the said auxiliary substances not only avoids damage to the antibody during freezing but also has a positive influence on the stability during long-term storage.

Example 3 elucidates the necessity of amino acids and surfactant in the preparation according to the invention. The use of sucrose as a builder alone leads to an unstable lyophilisate.

552780" 22880860

Example 4 describes variations of the amino acid component. It turns out that variation of the basic amino acids in the form of arginine or ornithine as well as the substitution of the basic amino acid by a neutral amino acid such as e.g. by leucine or by an acidic amino acid such as e.g. aspartic acid leads to a storage-stable preparation.

In example 5 the lyophilisation of a formulation containing sucrose, arginine and Tween 20 as well as phosphate buffer and sodium chloride is compared at various pH values (pH 5, pH 6.5 and pH 8). The data obtained show that it is possible to lyophilize within this pH range without impairing the stability.

If the said surfactant Tween 20 is replaced by a representative of the surface-active class of compounds polyoxyethylene-polyoxypropylene polymers (commercial name Pluronic®) as in example 6 this also results in an adequate stability of the preparation according to the invention.

Example 7 demonstrates the instability of a formulation containing mannitol as the builder as a substitute for sucrose, maltose or the amino sugar (see example 2).

If the sugar and the surfactant are omitted in the formulation the preparation becomes unstable as shown in example 8.

Although a combination of sugar (e.g. sucrose) and amino acids without surfactant in example 9 yields good results with regard to the parameters content and aggregates, the turbidity is, however, substantially

00308223 081299

increased compared to the formulations according to the invention containing sugar, amino acid and surfactant.

Example 10 shows that other monoclonal antibodies can also be stabilized with a combination of the sugar, amino acid and surfactant. The antibody anti-L-selectin is for example stable at a concentration of 7 mg in the lyophilisate. The lyophilization is carried out starting with a volume of 1 ml of an aqueous solution.

Investigative methods to determine stability

The lyophilized preparations were stored under defined storage conditions in the absence of light and subsequently analysed. The following test methods were used for the analyses.

OD280: Optical density at 280 nm. Photometric determination of the protein content, the UV absorbance is due to side chain chromophores such as tryptophan, tyrosine and phenylalanine residues. Specification: 95-105 %.

SE-HPLC: Size-exclusion high performance chromatography to determine aggregates. Specification: max. 2 %.

Measurement of turbidity: After reconstituting the lyophilisate the undiluted antibody solution was measured in a suitable turbidity photometer. Specification: max. 6 turbidity units.

00300223 081299

Example 1:

An aqueous stock solution of the MAB to HBV described above containing phosphate buffer and sodium chloride is prepared and examined. The concentration of the MAB is about 15 mg/ml.

Table 1a shows on the one hand the lability to freezing of the monoclonal antibody solution at various pH values at -20°C which already results in a decrease of the protein content after 4 weeks to 92.1 and 94.2 and 94.0 %. A decrease of the protein content is also observed on storage at 25°C. Under the storage conditions 4-8°C in a refrigerator the antibody is adequately stable over 9 months.

Tables 1b-1d show the stability data of the monoclonal antibody solution prepared at pH values 5, 6.5 and 8 at -20°C, 4-8°C and 25°C. This also shows that only a storage at 4-8°C is acceptable.

0030223 0030223 0030223

Table 1a: Change of the antibody content in the solution of active substance (10 mM phosphate buffer, 30 mM sodium chloride, water for injection purposes)

	pH 5			pH 6.5			pH 8		
Time	-20°C	4-8°C	25°C	-20°C	4-8°C	25°C	-20°C	4-8°C	25°C
start		> 99			> 99			> 99	
4 weeks	92.1	> 99	> 99	94.2	> 99	> 99	94.0	> 99	> 99
13 weeks	78.9	> 99	97.2	81.2	> 99	98.1	77.8	> 99	96.1
6 months	61.2	> 99	94.1	69.9	> 99	94.4	65.8	> 99	91.9
9 months	47.8	> 99	88.7	55.6	> 99	90.2	51.0	> 99	84.3

All data in %. The protein was determined by measuring the absorbance at 280 nm (OD 280).

Table 1b: Aggregate formation and turbidity values for the active substance solution of antibody, pH = 5

Times	-20°C		4-8°C		25°C	
	aggregates	turbidity	aggregates	turbidity	aggregates	turbidity
start	n.d.	1.5	n.d.	1.5	n.d.	1.5
4 weeks	aggregates	floccul.	n.d.	1.5	0.7 %	1.5
13 weeks	aggregates	floccul.	0.2 %	1.8	1.9 %	1.8
6 months	aggregates	floccul.	0.3 %	1.9	aggregates	9.9
9 months	aggregates	floccul.	0.6 %	2.1	aggregates	10.9

n.d.= not detectable

062180" 02200000

Table 1c): Aggregate formation and turbidity values for the active substance solution of antibody, pH = 6.5

Times	-20°C aggregates turbidity	4-8°C aggregates turbidity	25°C aggregates turbidity
start	n.d. 1.2	n.d. 1.2	n.d. 1.2
4 weeks	aggregates floccul.	n.d. 1.3	0.5 % 1.4
13 weeks	aggregates floccul.	0.2 % 1.4	1.8 % 1.7
6 months	aggregates floccul.	0.3 % 1.9	4.9 % floccul.
9 months	aggregates floccul.	0.6 % 2.1	9.3 % floccul.

Table 1d: Aggregate formation and turbidity values for the active substance solution of antibody, pH = 8

Times	-20°C aggregates turbidity	4-8°C aggregates turbidity	25°C aggregates turbidity
start	n.d. 1.4	n.d. 1.4	n.d. 1.4
4 weeks	2.0 % floccul.	0.3 % 1.5	0.74 % 1.7
13 weeks	2.8 % floccul.	0.5 % 1.8	1.95 % 2.1
6 months	3.7 % floccul.	0.6 % 1.9	3.0 % floccul.
9 months	5.4 % floccul.	0.8 % 2.1	4.3 % floccul.

Aggregates in % using SE-HPLC, turbidity in turbidity units (turbidity) using a turbidity photometer.

Example 2:

A solution of the monoclonal antibody to HBV according to example 1 was added to aqueous solutions of the

00308223 001 002 003 004 005 006 007 008 009 010 011 012 013 014 015 016 017 018 019 020 021 022 023 024 025 026 027 028 029 030 031 032 033 034 035 036 037 038 039 040 041 042 043 044 045 046 047 048 049 050 051 052 053 054 055 056 057 058 059 060 061 062 063 064 065 066 067 068 069 070 071 072 073 074 075 076 077 078 079 080 081 082 083 084 085 086 087 088 089 090 091 092 093 094 095 096 097 098 099 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

following sugars or amino sugars: sucrose (formulation 1), maltose (formulation 2) and N-methylglucosamine (formulation 3) containing arginine phosphate buffer and Tween 20 as the surfactant. The formulation is listed in example 2a. The final concentration of the MAB is 2 mg/ml. After adjusting the pH value with phosphoric acid to 6.5, the solutions were sterilized by filtration (0.22 μ m membrane filter) and filled into sterilized and depyrogenized injection bottles made of glass (hydrolytic class I) (filling volumes 1 ml) and lyophilized. After lyophilization the injection bottles were aerated with nitrogen, sealed automatically with stoppers in the freeze drying chamber and subsequently flanged.

The flanged injection bottles were stored in the absence of light for 4 to 13 weeks at various temperatures. After these periods the stability of the lyophilisates was examined with the described methods of examination.

Table 2a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form. 1 sucrose	100	n.d.	1.7	100	n.d.	1.6
form. 2 maltose	100	n.d.	1.6	100	n.d.	1.8
form. 3 N-methyl-glucosamine	100	n.d.	1.8	100	n.d.	1.5

Table 2b: Storage at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form. 1 sucrose	>99	n.d.	2.0	>99	n.d.	2.0
form. 2 maltose	>99	n.d.	1.9	>99	n.d.	2.1
form. 3 N-methyl-glucosamine	>99	n.d.	1.7	>99	n.d.	2.0

Legend:

I protein content in % with OD 280

II aggregates in % with SE-HPLC

III turbidity of the reconstituted solution in
turbidity units (dimensionless number)

n.d. not detectable (used in the same way in all further
tables)

Example 2a

In example 2a the formulation 1 from example 2 is prepared with 8 mg/ml antibody (= formulation 1a). It turns out that higher concentrations of up to 8 mg/1 ml antibody are adequately stable in this formulation.

09308223-081299

Compositions of formulations 1 and 1a:

	Formulation 1	Formulation 1a
MAB HBV	2.0 mg	8.0 mg
phosphate buffer	15 mM	15 mM
sodium chloride	30 mM	30 mM
sucrose	68.0 mg	58.0 mg
arginine	10.0 mg	10.0 mg
phosphoric acid	ad pH 6.5	ad pH 6.5
Tween 20	0.1 mg	0.1 mg
water for injection purposes	ad 1.0 ml	ad 1.0 ml

Table 3a: Stability data for formulation 1 and formulation 1a at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form.1: 2 mg/1ml	100	n.d.	1.7	100	n.d.	1.6
form.1a:8 mg/1ml	>99	n.d.	4.8	>99	n.d.	4.7

Table 3b: Stability data for formulations 1 and 1a at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form.1: 2 mg/1ml	>99	n.d.	2.0	>99	n.d.	2.0
form.1a:8 mg/1ml	>99	n.d.	4.7	>99	n.d.	5.5

- I protein content in % with OD 280
 II aggregates in % with SE-HPLC
 III turbidity of the reconstituted solution in
 turbidity units (dimensionless number)

0930623-0439
 66279-023060

Example 3

Comparison of formulations 1 and 4. Formulation 4 only contains sucrose as the builder and no arginine phosphate and no Tween 20. Formulation 4 is unstable.

	Formulation 1	Formulation 4
MAB HBV	2.0 mg	2.0 mg
phosphate buffer	15 mM	15 mM
sodium chloride	30 mM	30 mM
sucrose	68.0 mg	68.0 mg
arginine	10.0 mg	--
phosphoric acid or NaOH	ad pH 6.5	ad pH 6.5
Tween 20	0.1 mg	--
water for injection purposes	ad 1.0 ml	ad 1.0 ml

Table 4a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form.1: sucrose with arg. phos. and Tween 20	100	n.d.	1.7	>99	n.d.	1.6
form.4: sucrose without arg.phos. and Tween 20	98.3	1.6	6.1	96.0	4.3	9.5

Table 4b: Storage at 50°C:

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form.1:sucrose with arg.phos. and Tween 20	100	n.d.	2.0	>99	n.d.	2.0
form.4:sucrose without arg.phos. and Tween 20	96.0	4.2	8.5	89.8	10.1	10.9

Legend:

- I protein content in % with OD 280
- II aggregates in % with SE-HPLC turbidity of the reconstituted solution in turbidity units (dimensionless number)
- III turbidity of the reconstituted solution in turbidity units (dimensionless number)

Example 4

Variation of the amino acid component of the formulation. Formulations with basic, acidic and neutral amino acids are stable.

Composition of the formulations:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	35 - 70 mg
amino acid	variable
phosphoric acid or NaOH	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

09308223 081299

Table 5

	Amino acid
formulation 1	arginine (basic)
formulation 5	ornithine (basic)
formulation 6	leucine (neutral)
formulation 7	aspartic acid (acidic)

The pH value is adjusted by phosphoric acid or hydroxide solution.

Tables 6a - d

Examination results of formulations 1, 5, 6, 7 after storage for 4 and 13 weeks.

a) Table 6a, Formulation 1 (arginine):

	Storage period 25°C	4 weeks 50°C	Storage period 25°C	13 weeks 50°C
protein content % (OD 280)	100	>99	100	>99
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.
turbidity	1.7	2.0	1.6	2.0

b) Table 6b, formulation 5 (ornithine):

	Storage period 25°C	4 weeks 50°C	Storage period 25°C	13 weeks 50°C
protein content % (OD 280)	>99	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.
turbidity	1.9	1.9	2.0	2.1

c) Table 6c, formulation 6 (leucine):

	Storage period 25°C	4 weeks 50°C	Storage period 25°C	13 weeks 50°C
protein content % (OD 280)	>98	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	0.1	0.1
turbidity	2.2	2.4	2.8	2.7

d) Table 6d, formulation 7 (aspartic acid):

	Storage period 25°C	4 weeks 50°C	Storage period 25°C	13 weeks 50°C
protein content % (OD 280)	>98	>98	>98	>98
aggregates % (SE-HPLC)	n.d.	n.d.	0.1	0.1
turbidity	2.7	2.7	3.4	4.0

662780" 22280660

Example 5

Example 5 contains formulation 1 at various pH values, the lyophilisates are prepared as described in example 2, the pH of the solution of auxiliary substances and of the product solution was adjusted before lyophilisation with 85 % phosphoric acid.

Formulation:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68 mg
arginine	10 mg
phosphoric acid	ad pH 5; 6.5; 8
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

The lyophilisates were prepared with the pH values shown in table 7.

After flanging the injection bottles these were stored in the absence of light under defined temperature conditions. After storage periods of 4 weeks and 13 weeks the samples were analysed (protein content in %: OD 280, aggregates in %: SE-HPLC, turbidity). The formulation was stable at all pH values.

09300223 "081299
062180" 22280260

Table 7:

	pH
formulation 8	5
formulation 9 (ident. with 1)	6.5
formulation 10	8

Table 8a: Storage at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
formulation 8	100	n.d.	1.9	>99	n.d.	2.3
formulation 9(=1)	100	n.d.	1.7	100	n.d.	1.6
formulation 10	>99	n.d.	2.3	>99	n.d.	2.6

Table 8b: Storage at 50°C

	Storage 13 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
formulation 8	>99	n.d.	2.2	>99	n.d.	2.3
formulation 9(=1)	>99	n.d.	2.0	>99	n.d.	2.0
formulation 10	>98	n.d.	2.5	>98	n.d.	2.6

Legend:

- I protein content in % with OD 280
- II aggregates in % with SE-HPLC
- III turbidity of the reconstituted solution in
turbidity units (dimensionless number)

09308223 08199 662780 2280260

Example 6

The formulation described in the following containing the surfactant Pluronic F 68 instead of Tween 20 was prepared as described above.

The storage and examination of stability was carried out in an analogous manner to that of the other examples.

Formulation 11:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	48.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Pluronic F 68	0.1 mg
water for injection purposes	ad 1.0 ml

Formulation 1 is chosen as a comparison and is identical to formulation 11 except for Tween 20 instead of Pluronic F 68. Both formulations were stable.

Table 9: Stability data of the formulation containing the surfactants Pluronic F 68 and Tween 20.

	formulation 11				formulation 1			
	storage period		storage period		storage period		storage period	
	4 weeks		13 weeks		4 weeks		13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	>98	>98	>98	>98	100	>99	100	>99
aggregates % (SE-HPLC)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
turbidity	1.9	1.9	2.5	2.2	1.7	2.0	1.6	2.0

Example 7:

The formulation 12 described in this example essentially corresponds to formulation 1 except that mannitol was used instead of sucrose as a builder. It can be seen that the mannitol formulation is unstable.

Formulation 12:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
mannitol	25.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

Table 10: Stability data of the formulations containing the builder mannitol (formulation 12) and sucrose (formulation 1)

	formulation 12				formulation 1			
	storage period		storage period		storage period		storage period	
	4 weeks		13 weeks		4 weeks		13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	96.2	91.8	94.0	84.5	100	>99	100	>99
aggregates % (SE-HPLC)	3.6	8.4	5.8	15.9	n.d.	n.d.	n.d.	n.d.
turbidity	3.2	6.9	4.9	13.2	1.7	2.0	1.6	2.0

Example 8

Further evidence for the necessity of the combination of sugar, amino acid and surfactant is given by comparing formulation 1 which contains all listed components with formulation 13 composed of antibody, phosphate buffer, sodium chloride and arginine phosphate. The aggregate formation is increased and the turbidity values become worse without sugar and surfactant.

Formulation 13

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
arginine	35.0 mg
phosphoric acid	ad pH 6.5
water for injection purposes	ad 1.0 ml

Table 11: Stability data for formulation 13 (without sucrose and Tween 20 only with arginine phosphate as builder) and formulation 1

	formulation 13				formulation 1			
	storage period		storage period		storage period		storage period	
	4 weeks		13 weeks		4 weeks		13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	97.6	94.9	95.8	89.0	100	>99	100	>99
aggregates % (SE-HPLC)	2.6	4.5	4.0	10.7	n.d.	n.d.	n.d.	n.d.
turbidity	2.9	4.5	3.8	12.3	1.7	2.0	1.6	2.0

Example 9

Although a stable formulation is obtained without surfactant (Tween 20) and only with sucrose and arginine, the turbidity values worse (formulation 14).

Formulation 15:

MAB HBV	2.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
water for injection purposes	ad 1.0 ml

Table 12: Stability data of formulation 14 and formulation 1

	formulation 14				formulation 1			
	storage period		storage period		storage period		storage period	
	4 weeks		13 weeks		4 weeks		13 weeks	
	25°C	50°C	25°C	50°C	25°C	50°C	25°C	50°C
protein content % (OD 280)	>99	>98	>98	>98	100	>99	100	>99
aggregates % (SE-HPLC)	0.2	0.3	0.5	1.3	n.d.	n.d.	n.d.	n.d.
turbidity	3.4	4.8	8.8	13.3	1.7	2.0	1.6	2.0

Example 10

The following table shows the components of formulation 15. The antibody used is anti-L-selectin. The data shown in table 13a on the examination of stability show that the formulation used enables an adequate stabilization.

Composition of formulation 15:

	Formulation 15
anti-L-selectin	7.0 mg
phosphate buffer	15 mM
sodium chloride	30 mM
sucrose	68.0 mg
arginine	10.0 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

Table 13a: Stability data for formulation 15 at 25°C

	Storage 4 weeks at 25°C			Storage 13 weeks at 25°C		
	I	II	III	I	II	III
form. 15:7 mg/1ml	>99	n.d.	2.5	>99	n.d.	2.9

Table 13b: Stability data for formulation 15 at 50°C

	Storage 4 weeks at 50°C			Storage 13 weeks at 50°C		
	I	II	III	I	II	III
form. 15:7 mg/ml	99	n.d.	4.1	99	n.d.	5.2

I protein content in % with OD 280

II aggregates in % with SE-HPLC

III turbidity of the reconstituted solution in
turbidity units (dimensionless number)

Example 11

Stabilization of the antibody anti-L-NGFR (anti-L-nerve-growth-factor-receptor)

09308223 084299 66280 228060

Formulation 16:

A lyophilisate with the following formulation (analogous to formulation 1) is prepared:

	Formulation 16
anti-L-NGFR	0.25 mg
phosphate buffer	15 mM
sucrose	75 mg
arginine	10 mg
phosphoric acid	ad pH 6.5
Tween 20	0.1 mg
water for injection purposes	ad 1.0 ml

The lyophilisate of anti-L-NGFR is prepared analogously to the preparation of the MAB-HBV and anti-L-selectin lyophilisates.

An aqueous solution containing the additives sugar, amino acid and surfactant at pH 5 to 8 is admixed with a solution of the anti-L-NGFR in a phosphate buffer. The phosphate salts are added in such amounts that the previously defined concentrations are obtained. Subsequently it is sterilized by filtration and the solution prepared in this manner is lyophilized. After lyophilisation one obtains an optically perfect lyophilisation cake. The antibody anti-L-NGFR remains stable. After reconstitution of the lyophilisate with water for injection purposes a clear solution is obtained.